BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : G01N 1/28

A1

(11) International Publication Number: WO 86/ 06479

(43) International Publication Date: 6 November 1986 (06.11.86)

(21) International Application Number: PCT/SE86/00185

(22) International Filing Date: 22 April 1986 (22.04.86)

(31) Priority Application Number: 8501959-4

(32) Priority Date: 23 April 1985 (23.04.85)

(33) Priority Country: SE

(71)(72) Applicant and Inventor: RENVALL, Henrik, Gerhard [FI/SE]; Ehrlangengatan 8, S-632 30 Eskilstuna (SE).

(74) Agent: JOHANSSON, Lars, E.; LKB-Produkter AB, Box 305, S-161 26 Bromma (SE).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.
With amended claims.
In English translation (filed in Swedish).

(54) Title: METHOD OF IMPREGNATING A TISSUE SPECIMEN WITH PARAFFIN

(57) Abstract

In a method of impregnating a tissue specimen with paraffin either directly after an optional treatment of the tissue specimen with a fixing agent or after the tissue specimen optionally has been treated with a fixing agent and/or a dehydrating agent an immersion of the tissue specimen into a bath of liquid paraffin takes place. The bath is kept below a predetermined temperature at which thermolabile components and properties of the tissue specimen undergo changes. The pressure of the bath is then lowered from atmospheric pressure to a predetermined pressure at which water present in the tissue specimen and/or, when appropriate, the dehydrating agent vaporizes, the pressure of the bath then being increased again to atmospheric pressure.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GA**	Gabon	**MR**	Mauritania
AU	Australia	**GB**	United Kingdom	**MW**	Malawi
BB	Barbados	**HU**	Hungary	**NL**	Netherlands
BE	Belgium	**IT**	Italy	**NO**	Norway
BG	Bulgaria	**JP**	Japan	**RO**	Romania
BR	Brazil	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SN**	Senegal
CH	Switzerland	**LI**	Liechtenstein	**SU**	Soviet Union
CM	Cameroon	**LK**	Sri Lanka	**TD**	Chad
DE	Germany, Federal Republic of	**LU**	Luxembourg	**TG**	Togo
DK	Denmark	**MC**	Monaco	**US**	United States of America
FI	Finland	**MG**	Madagascar		
FR	France	**ML**	Mali		

Method of impregnating a tissue specimen with paraffin.

TECHNICAL FIELD

The invention refers to a method of impregnating a tissue specimen with paraffin.

BACKGROUND ART

Such methods are known from GB 1 536 422 and GB-A- 2 065 912.

In both these known methods the tissue specimens are treated with a dehydrating agent, alcohol and acetone, respectively, before the tissue specimens are immersed in a bath of liquid paraffin.

After the tissue specimens have been immersed in the paraffin bath, the pressure of the bath is lowered so much that the respective dehydrating agent is vaporized and thereby evaporates from the tissue specimens as well as from the paraffin bath, the pressure of the bath then being increased again to atmospheric pressure.

From GB 1 536 422 it is apparent that the operating temperature is 65°C, while no temperature information can be found in GB-A-2 065 912. However, it could be assumed that the operating temperature also in the last mentioned case is about 60-65°C.

However, it has been found that a temperature higher than about 45-47°C destroys the structure of the tissue specimens and also has a shrivelling effect on the tissues. It has also been found that proteins and other thermolabile tissue components as well as thermolabile properties are changed at higher temperature in such a way that antigen structures cannot be recognized to a sufficient extent in a subsequent histochemical tissue diagnosis. Moreover, membranes and organelles become shrivelled and are destroyed.

DISCLOSURE OF INVENTION

An object of the invention is to provide a method of impregnation that eliminates the disadvantages of the methods known hitherto.

Another object of the invention is to provide a method of impregnation in which the treatment with a dehydrating agent can be eliminated.

This is attained by the method according to the invention in that the tissue specimen is immersed, either directly after an optional treatment of the tissue specimen with a fixing agent or after the tissue specimen optionally has been treated with a fixing agent and/or a dehydrating agent, in a bath of liquid paraffin which is kept below a predetermined temperature at which thermolabile components and properties of the tissue specimen undergo changes, and that thereafter, the pressure of the bath is lowered from atmospheric pressure to a predetermined pressure, at which water and/or, when appropriate, the dehydrating agent vaporizes. Then the pressure of the bath is increased again to atmospheric pressure.

PREFERRED EMBODIMENTS

When carrying out a histochemical diagnosis, normally a tissue specimen is first treated with a fixing agent, usually formalin, and thereafter with a dehydrating agent, e.g. methanol, ethanol or acetone, before the tissue specimen is immersed in a bath of liquid paraffin. In this bath the tissue specimen is impregnated with paraffin. The tissue specimen impregnated in this way with paraffin is then embedded in a paraffin block which can be cut by means of a microtome knife to obtain thin cuts (1-5 µm thick) which can thereafter be examined in a microscope after the paraffin has been removed and after dyeing.

According to the invention it is suggested that after an optional treatment of the tissue specimen with a fixing

agent, the tissue specimen is directly immersed into the paraffin bath. For this bath a paraffin having a melting point of about 37-44°C has been chosen and the bath is kept at a temperature of about 47°C at the most, i.e. below the temperature at which it has been observed that thermolabile components and properties of the tissue specimen begin to change. It should be pointed out that this temperature can vary somewhat depending on which type of tissue specimen one wants to examine.

According to the invention the pressure of the paraffin bath is thereafter lowered from atmospheric pressure to at least about 76 mm Hg, i.e. the pressure at which water boils at a temperature of about 46°C.

In this way, water present in the tissue specimen will vaporize and evaporate from the tissue specimen as well as from the paraffin bath.

Then, the pressure of the bath is increased again to atomospheric pressure. The lowering of the pressure as well as the pressure increase must not take place too abrupt. The tissue material and cells present therein can burst through sudden pressure changes during the pressure lowering phase and can be compressed during the pressure increasing phase.

According to another embodiment of the invention the tissue specimen can also be treated with a dehydrating agent, for example methanol, ethanol or acetone, before the sample is immersed into the paraffin bath which also in this case is kept below the temperature at which thermolabile components and properties of the tissue specimen are changed.

When acetone is used as a dehydrating agent, the pressure of the paraffin bath has to be lowered to at least about 510 mm Hg, i.e. the pressure at which acetone vaporizes at a temperature of about 45°C.

Thus, the acetone will vaporize and evaporate from the tissue specimen as well as from the paraffin bath.

To ensure that any remaining water also will evaporate from the tissue specimen, the pressure of the bath must also in this case be lowered to at least 76 mm Hg.

By the paraffin impregnating method according to the invention it has been found that the quality of the tissue specimens is improved compared to paraffin impregnating methods known hitherto.

CLAIMS

1. Method of impregnating a tissue specimen with paraffin, characterized in that either directly after an optional treatment of the tissue specimen with a fixing agent or after the tissue specimen optionally has been treated with a fixing agent and/or a dehydrating agent, the tissue specimen is immersed in a bath of liquid paraffin which is kept below a predetermined temperature at which thermolabile components and properties of the tissue specimen undergo changes, that the pressure of the bath is then lowered from atmospheric pressure to a predetermined pressure at which water present in the tissue specimen and/or, when appropriate, the dehydrating agent vaporizes, whereupon the pressure of the bath is increased to atmospheric pressure.

2. Method according to claim 1, characterized in that the temperature of the bath is kept at about 47°C at the most.

3. Method according to claim 1, characterized in that the pressure of the bath is lowered to at least about 510 mm Hg.

4. Method according to claim 3, characterized in that the pressure of the bath is lowered to at least about 76 mm Hg.

AMENDED CLAIMS

[received by the International Bureau on 12 September 1986 (12.09.86);
original claims 1—4 replaced by new claim 1 (1 page)]

Method of impregnating a tissue specimen with paraffin by immersing the tissue specimen in a bath of liquid paraffin which is kept at a temperature of about 47°C at the most, characterized in that the tissue specimen is immersed into the bath directly after an optional treatment of the tissue specimen with a fixing agent, that the pressure of the bath in then lowered from atmospheric pressure to at least about 75 mm Hg to vaporize water present in the tissue specimen, whereupon the pressure of the bath is increased to atmospheric pressure.

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE86/00185

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC4

G 01 N 1/28

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
IPC 4	G 01 N 1/28, /30
US Cl	424:3; 118:10, 11

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT*

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X	CH, B, 456 049 (THE TECHNICON COMPANY, INC.) 15 July 1968 See column 10 lines 12-28, column 11 lines 20-56 and column 12 lines 1-9.	1-4
A	GB, B, 1 536 422 (SHANDON SOUTHERN PRODUCTS LIMITED) 20 December 1978	
A	GB, A, 2 065 912 (C. THOMAS) 1 July 1981	

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1986-07-04	1986 -07- 10
International Searching Authority	**Signature of Authorized Officer**
Swedish Patent Office	May Hallne

Form PCT/ISA/210 (second sheet) (January 1985)

L.E